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ABSTRACT

A moderate cold exposure has been used as a tool in inducing a higher food intake in experiments dealing with amino acid imbalances in the rat. The data indicate that the rats kept at 25° C suffer a severe metabolic disorder after ingesting amino acid imbalanced diets. In contrast, the animals kept at 7° C readily consumed the imbalanced diets and, consequently, grew as well as the controls. The activity of the glutamic-oxalacetic and the glutamic-pyruvic transaminase were found to be increased as a result of a cold exposure. It is suggested that a moderate cold stress is an effective agent in correcting and overcoming amino acid imbalances.

PUBLICATION REVIEW

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INTERRELATIONSHIPS OF COLD EXPOSURE AND AMINO ACID IMBALANCES

SECTION 1. INTRODUCTION

From the recent work concerning the effects of amino acid imbalances, it is obvious that the main obstacle in studying these phenomena is the refusal of animals to consume voluntarily adequate amounts of the imbalanced diet, which in turn causes a major difficulty in interpreting and extrapolating the experimental data. In an effort to overcome this problem, and thus to approach the normal physiological state of the animal, such techniques as forced-feeding (Deshpande et al, 1958), spaced-feeding (Kumta et al, 1958) or insulin injections (Spolter and Harper, 1961) have been used as a means of inducing a higher food intake. However, these techniques proved either unsatisfactory or even detrimental to the animal and the results obtained may be subjected to an alternative explanation.

In view of the finding in this laboratory (Vaughan and Vaughan, 1957, 1959, 1960, 1961) that appetite of cold exposed rats, while characteristically depressed in vitamin deficiencies, was simultaneously stimulated by the low environmental temperature, it seemed to us advantageous to use cold exposure as a tool to induce voluntarily a higher intake of imbalanced diets in studying amino acid imbalances in the rat. The results of such experiments are presented in this work.

SECTION 2. EXPERIMENTAL

Male, Sprague-Dawley rats, ranging in weight from 160 to 200 gm, were used in all experiments. In each experiment they were divided into two groups. One group was placed in a cold room held at 7° C, while the other group remained in an animal room at 25° C. All rats were housed in individual wire cages and both the diets and water were offered on an ad libitum basis. The animals and food refusals were weighed every second day.

The basal low-protein fibrin or casein diets and the imbalancing amino acid mixture were patterned after those used by Kumta and Harper (1960). The basal diet consisted of 6% fibrin, 85% sucrose (or 9% crude casein and

82% sucrose), 5% corn oil, and 4% U. S. P. salt mixture No. II. The vitamin mixture supplied 2, 000 units of vitamin A, 222 units of vitamin D, 11. 1 mg of alpha-tocopherol and the following in mg per 100 gm of diet: ascorbic acid 100; inositol 11. 1; choline chloride 166. 5; menadione 5; p-amino-benzoic acid 11. 1; niacin 10; pyridoxine hydrochloride 2. 22; riboflavin 2. 22; thiamin hydrochloride 2. 22; Ca-pantothenate 40. 3; also 44 mg of biotin, 200μg of folic acid and 3μg of vitamin B_{12}.

The imbalancing amino acid mixture consisted of: DL-methionine 0. 4%; DL-phenylalanine 0. 6%; L-leucine 0. 4%; DL-isoleucine 0. 4%; DL-valine 0. 7%; L-lysine HCl 0. 6%; L-arginine HCl 0. 2%; L-tryptophan 0. 2%; DL-threonine 0. 4%; L-glutamic acid 1. 0%; and L-histidine HCl 0. 4%. The imbalanced diet was created by adding one or two amino acids, or a mixture of amino acids lacking one or several amino acids, to the basal diets. All substitutions were made at the expense of sucrose in the basal diet. At the end of 21 days the animals were sacrificed by decapitation and the blood collected in centrifuge tubes containing a drop of heparin. The liver was immediately excised, chilled in chipped ice and assayed for arginase, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT).

Arginase determination was carried out according to the procedure of Brown and Cohen (1959) with the following modifications: 10% liver homogenates were prepared in glass double-distilled water and 0. 2 ml of the 1:1250 water diluted homogenate was added to the incubation mixture consisting of 0. 2 ml L-arginine 0. 85 M, pH 9. 5; 0. 2 ml glycine 0. 50 M, pH 9. 5; 0. 2 ml $MnCl_2$, 0. 005 M and of 1. 2 ml glass double-distilled water. Incubations were carried out in 10 ml beakers at 38° C with shaking for 30 minutes. Reactions were stopped with 2 ml of 0. 5M perchloric acid, and after centrifugation the clear supernatant was analyzed for urea with 1-phenyl-1:2-propanedione-2-oxime according to the method of Archibald (1944) as modified by Ratner (1955). To obtain the value for endogenous urea, the enzyme in the incubation mixture was inactivated with perchloric acid and the incubation was carried out as usual. The value for endogenous urea was subtracted from the experimental urea value. Both the incubations and the urea determinations were carried out on at least duplicate samples.

GOT and GPT were measured by following the oxidation of DPNH with a Beckman DK recording spectrophotometer according to procedures similar to those used by Wroblewski and LaDue (1956) and LaDue et al (1954). Aliquots of tissues were analyzed for nitrogen content by acid digestion and the nesslerization procedure. Since there was no difference in the nitrogen content of the livers, the results of the enzyme assays were calculated as μM of products per minute per 100 gm of body weight. It was felt that this method of expressing the activity more closely reflects the animals' total ability to produce a given product in relation to their needs.

The levels of plasma and urinary amino nitrogen were determined spectrophotometrically by the ninhydrin method. Since urea interferes with the color development, it was separated from the amino acids in the deproteinized plasma or the urine by paper chromatography in a n-butanol:95% ethanol: water (4:1:1) system. The amino acids were eluted with water and the color developed according to the procedure of Saifer et al (1960). Individual urine collections were carried out in standard metabolism cages over a 24-hour period and the urine samples were kept frozen until analyzed.

SECTION 3. RESULTS AND DISCUSSION

The design and results of the first experiment are presented in Table I. The data show that an addition of 5% L-leucine to the casein basal diet caused a severe retardation of growth and a depression in appetite both in the warm and the cold groups. It should be noted that despite a highly toxic level of leucine in the diet, the cold exposed animals increased their food consumption almost 75% over the corresponding warm group, thus also proportionally increasing the absolute daily intake of this amino acid from approximately 395 mg to 690 mg. However, such an increment in leucine intake had no additional adverse effect on growth.

TABLE I

EFFECT OF COLD EXPOSURE AND AMINO ACID IMBALANCE ON GROWTH AND FOOD CONSUMPTION OF RATS (EXPERIMENT 1)

		ΔW gm/3 Weeks		Food Intake gm/day	
Group	Diet	Warm	Cold	Warm	Cold
1	9% Casein	61.1 ± 2.4*	50.8 ± 6.3	11.9 ± 0.39	17.3 ± 0.23
2	9% Casein + 5% L-leucine	-7.1 ± 3.0	-3.9 ± 3.2	7.9 ± 0.32	13.8 ± 0.15
3	6% Fibrin	42.8 ± 2.8	39.6 ± 3.8	11.7 ± 0.45	17.1 ± 0.15
4	6% Fibrin + amino acid mixture -histidine	22.7 ± 2.7	39.0 ± 2.7	8.6 ± 0.28	16.7 ± 0.31

* Standard error of the mean for 7 rats

A similar effect of cold on appetite and growth can be seen in Group 4, in which an amino acid imbalance was induced by adding an amino acid mixture lacking histidine to the fibrin basal diet. This imbalanced diet caused a considerable decrease both in food intake and the rate of growth of the warm rats, thus confirming similar findings of Kumta and Harper (1960).

In contrast, the cold exposed animals readily consumed the imbalanced diet and, consequently, grew as well as the controls (Group 3). However, in another experiment (Table III, Group 7) the histidine-lacking diet did not cause any growth depression when offered to the warm rats, although a marked growth response was again obtained in the cold group. Thus, it appears that an omission of histidine from the amino acid mixture employed produces only a borderline imbalance which can be readily corrected by cold exposure.

An imbalance produced by omitting isoleucine from the amino acid mixture was studied in the second experiment (Table II, Group 2). Again, the lack of isoleucine produced a pronounced growth retardation and a depression of appetite in the warm group, an effect reported previously by Kumta and Harper (1960). However, these adverse effects were overcome to a great extent by the cold exposure. The higher food intake of the cold exposed animals is reflected in the higher levels of the amino acid nitrogen both in the plasma and in the urine.

Incorporation of 0.4% DL-methionine and 0.6% DL-phenylalanine in the fibrin diet (Group 3) produced a severe imbalance in the warm group, which is in agreement with the findings of Deshpande et al (1958). On the other hand, these two amino acids failed to produce any retarding effect on growth and appetite in the cold exposed animals, despite the fact that their absolute intake was increased by 90 per cent. It should be pointed out that rats forced-fed a similar diet by Deshpande et al (1958) died within two or three days, apparently due to their inability to metabolize efficiently the diet. In contrast, the cold exposed animals not only tolerated a higher intake of the two imbalancing amino acids, but grew as well as the controls.

The leucine-isoleucine antagonism, as defined and reported by Harper et al (1955) and Benton et al (1956), was studied in the third experiment (Table III). When either isoleucine (Group 2) or isoleucine and valine (Group 4) were omitted from the amino acid mixture, a severe growth depression occurred in the two warm groups. Furthermore, an omission of leucine together with isoleucine (Group 3) or leucine together with isoleucine and valine (Group 5) restored normal growth, showing that an excess of leucine is responsible for some metabolic alteration affecting appetite, which in turn leads to a depression in growth. Interestingly, this phenomenon was completely overcome by the cold exposure. The cold exposed animals were

TABLE II

EFFECT OF COLD EXPOSURE AND AMINO ACID IMBALANCE ON GROWTH, FOOD CONSUMPTION AND URINARY AND PLASMA AMINO NITROGEN OF RATS (EXPERIMENT 2)

Group	Diet	ΔW gm/3 weeks		Food Intake gm/day		Urinary NH_2-N mg/24 hrs		Plasma NH_2-N mg/24 hrs	
		Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold
1	6% Fibrin	42.4 ± 1.5[1]	50.8 ± 5.8	12.2 ± 0.58	19.0 ± 0.72	3.99 ± 0.21	8.89 ± 0.25[3]	7.81 ± 0.32	10.77[3] ± 0.46
2	6% Fibrin +amino acid mixture -DL-isoleucine	19.3 ± 3.0	36.7 ± 4.3	9.5 ± 0.32	17.6 ± 0.67	18.98 ± 0.36[2]	25.78 ± 0.41[2]	9.34[2] ± 0.36	10.68 ± 0.27
3	6% Fibrin +0.4% DL methionine +0.6% DL-phenylalanine	28.4 ± 4.6	58.4 ± 3.5	10.4 ± 0.42	19.8 ± 0.14	10.88 ± 0.62[2]	16.12 ± 0.57[2]	8.44 ± 0.29	10.26 ± 0.35

[1] Standard error of the mean for 7 rats

[2] Difference from fibrin group ($p < 0.05$)

[3] Difference from warm group - same diet ($p < 0.05$)

TABLE III

EFFECT OF COLD EXPOSURE AND THE LEUCINE-ISOLEUCINE ANTAGONISM ON GROWTH, FOOD CONSUMPTION AND GOT, GPT AND ARGINASE ACTIVITIES (EXPERIMENT 3)

Group		1	2	3	4	5	6	7
Diet		6% Fibrin	6% Fibrin + amino acid mix -isoleucine	6% Fibrin + amino acid mix -isoleucine -leucine	6% Fibrin + amino acid mix -isoleucine -valine	6% Fibrin + amino acid mix -isoleucine -leucine -valine	6% Fibrin + complete amino acid mix	6% Fibrin + amino acid mix -histidine
ΔW	Warm	35.8 ± 1.4[1]	18.7 ± 3.3	37.2 ± 2.2	18.4 ± 2.6	32.8 ± 6.6	85.7 ± 5.4	42.6 ± 2.8
(gm/3 weeks)	Cold	41.3 ± 5.2	34.5 ± 6.0	44.5 ± 4.3	35.6 ± 3.0	39.3 ± 7.0	76.3 ± 5.0	58.8 ± 4.8
Food intake	Warm	13.4 ± 0.38	11.7 ± 0.52	12.8 ± 0.54	11.7 ± 0.46	14.5 ± 0.87	15.1 ± 0.36	14.0 ± 0.60
(gm/24 hrs)	Cold	18.2 ± 0.53	18.0 ± 0.79	18.9 ± 0.64	17.7 ± 0.59	19.3 ± 0.57	20.8 ± 0.22	19.8 ± 0.39
GOT (μM/min/10 gm BW)								
	Warm	510 ± 54	718[2] ± 35	788[2] ± 55	698[2] ± 66			
	Cold	753[3] ± 54	856 ± 57	814 ± 73	829 ± 86			
GPT (μM/min/100 gm BW)								
	Warm	129 ± 9.7	194[2] ± 10.3	195[2] ± 9.3	191[2] ± 20.2			
	Cold	171[3] ± 8.0	229 ± 30.2	196 ± 17.9	191 ± 18.5			
Arginase (μM/min/100 gm BW)								
	Warm	526 ± 29	692[2] ± 36	721[2] ± 42	678[2] ± 29			
	Cold	652[3] ± 38	718 ± 45	695 ± 45	701 ± 31			

[1] Standard error of the mean for 7 rats
[2] Difference from fibrin group ($p<0.05$)
[3] Difference from warm group - same diet ($p<0.05$)

able to increase food intake and performed as well as either of the controls (Group 1). As expected, the complete amino acid mixture added to the fibrin basal (Group 6) supported an excellent rate of gain.

It will be noted that the activity of the two transaminases and of the arginase was higher in the cold controls than in the warm controls, possibly as a result of an increased food intake in the cold. An omission from the amino acid mixture of either isoleucine alone (Group 2) or isoleucine plus leucine (Group 3) or isoleucine plus valine (Group 4) resulted in a uniform increase in the activity of all three enzymes. The enzyme activity in this case was not, however, further affected by the environmental temperature.

Although the basic mechanism concerning the phenomena presented herein remains to be determined, the results of these experiments indicate that a moderate cold stress is an effective agent in correcting and overcoming amino acid imbalances. A warm rat, which suffers a severe metabolic disorder when it consumes a diet containing an imbalanced amino acid mixture, can effectively metabolize and utilize it for tissue synthesis when exposed to cold. The data of Kumta et al (1958) indicate that feeding an imbalanced diet either increases the breakdown of tissue proteins or that an imbalanced diet is not properly utilized for the protein synthesis. From the data presented in the present work it would appear that the cold exposed animals are able to use an imbalanced diet for the formation of the tissue proteins. The cold exposed animals could possibly accomplish this step by catabolizing preferentially the imbalancing portion of the amino acid mixture and utilizing it for heat production. The remaining balanced portion of the mixture could be then effectively utilized for the protein synthesis.

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2. Amino Acids
3. Rats

I. Project 8238-03
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